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Gene-nutrient interactions on metabolic diseases: Findings from the GeNuIne Collaboration

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Abstract:

This article describes how the British Nutrition Foundation Drummond pump priming award was used to initiate a large scale collaborative project called *Gene-Nutrient Interactions (GeNuIne) Collaboration*, the main objective of which is to investigate the effect of gene-nutrient interactions (nutrigenetics) on metabolic disease outcomes using population-based

studies from various ethnic groups. The article also provides a summary of gene-diet interaction studies, performed in developing countries as part of this collaborative project, and gives an overview of how nutrigenetic findings can be translated to personalised and public health approaches for the prevention of metabolic diseases such as obesity and type 2 diabetes.

Key words: Obesity, type 2 diabetes, nutrigenetics, gene-nutrient interactions, personalised nutrition, GeNuIne Collaboration

Introduction

Metabolic diseases such as obesity and type 2 diabetes are world-wide public health problems afflicting various populations and are caused generally by the interaction of overconsumption of energy, sedentary lifestyle and genetic susceptibility (Vimaleswaran & Loos 2010). Dietary factors play an important role in the development of metabolic diseases, but this relationship may differ from country to country because of the variation in the food being consumed in different parts of the world. Studies have shown that under-nutrition during the perinatal period can lead to an 85% reduction in expression of brown fat biomarkers and genes involved in citric acid cycle and fatty acid oxidation (Kozak 2012), providing evidence for interactions between genes and nutrients in the development of obesity. Although several studies have examined the interactions between genes and macronutrients on obesity, the findings have been inconsistent because of two main challenges: i) genetic heterogeneity and ii) insufficient sample size, and hence, it is unable to develop a personalised diet for each ethnic group. To address these issues, the *Gene-Nutrient Interactions* (*GeNuIne*) *Collaboration* has been initiated to investigate the effect of gene-nutrient interactions on metabolic diseases using population-based studies in various ethnic groups.

The prevalence of metabolic diseases such as type 2 diabetes and obesity are increasing at an alarming rate in both developing and developed countries. Hence, it is crucial to investigate the interactions between genetic and dietary factors to understand the role of diet in the genetic predisposition towards type 2 diabetes and obesity and to facilitate personalised nutrition (Joost *et al.* 2007). Given that the genetic make-up varies from one individual to another, it is important to explore gene-diet interactions in other ethnicities as well, which will eventually enable us to personalise diet according to each ethnic sub-group. GeNuIne Collaboration aims to meet this objective by implementing gene-diet interaction studies in developing countries.

British Nutrition Foundation Drummond Pump Priming award

The *GeNuIne Collaboration* was initiated through funds from the British Nutrition Foundation (BNF) Drummond Pump Priming award, which is awarded to talented early-to-mid career academics in human nutrition to undertake the pilot work needed to generate data that can be used as the basis for conducting a large-scale study. To establish collaborations with researchers in developing countries, travel grants from the British Council Researcher Links were obtained. Through these travel grants, collaborations were established in India, Brazil, Morocco, Turkey, Thailand, Sri Lanka, Indonesia and Pakistan. The Pump Priming award was used to carry out genetic analyses in the UK and other countries. Although gene-nutrient interactions have been examined extensively in the Western population, very few studies have been carried out in the developing countries and, hence, the *GeNuIne Collaboration* has been established to address this missing gap in human nutrition in these countries.

Findings from GeNuIne Collaboration

Impact of genes and diet on metabolic diseases in an Asian Indian population

India has the second largest number of people with type 2 diabetes in the world, with 62.4 million people living with this condition according to the *Indian Council of Medical Research-INdia DIABetes* (*ICMR-INDIAB*) Study (Anjana *et al.* 2011). Asian Indians have unique clinical and biochemical characteristics that are collectively referred to as the 'South Asian' or 'Asian Indian Phenotype' (higher waist circumference, higher levels of total and visceral fat, hyperinsulinemia and insulin resistance) (Mohan & Deepa 2006), which confers

an increased susceptibility to type 2 diabetes and premature cardiovascular disease. Although there is a strong genetic component to the higher prevalence of metabolic diseases amongst Asian Indians (Vimaleswaran *et al.* 2008; Vimaleswaran *et al.* 2006; Vimaleswaran *et al.* 2010b; Vimaleswaran *et al.* 2011; Vimaleswaran *et al.* 2010a; Vimaleswaran *et al.* 2005), unhealthy diet and physical inactivity have also been shown to contribute (Mohan *et al.* 2014; Anjana *et al.* 2014).

Given the situation among Asian Indians, our most recent study (Vimaleswaran *et al.* 2016) examined whether dietary intake and physical activity levels modified associations between two commonly studied fat mass and obesity–associated gene (*FTO*) single nucleotide polymorphisms (SNPs) and obesity-related traits and type 2 diabetes in 1,618 Asian Indians. In this study, the participants were recruited from the urban component of the *Chennai Urban Rural Epidemiology Study* (*CURES*), a cross-sectional epidemiological study conducted on a representative sample of the population of Chennai (formerly Madras city) in Southern India (Deepa *et al.* 2003). Dietary intakes were assessed using a previously validated and published (Sudha *et al.* 2006) interviewer administered semi-quantitative food frequency questionnaire (FFQ) containing 222 food items to estimate food intake over the past year. Physical activity was estimated using a previously validated self-report questionnaire (Mohan *et al.* 2007). The *CURES* study was funded by Lady Tata Memorial Trust, Mumbai, India and the Chennai Wellingdon Corporate Foundation, Chennai, India.

Of the several obesity-related genes, the fat mass and obesity–associated (*FTO*) gene variants have been shown to be consistently associated with obesity traits in several populations and has been the strongest common genetic predictor of obesity known so far (Vimaleswaran *et al.* 2012b; Vimaleswaran *et al.* 2012a; Bradfield *et al.* 2012). Our study (Vimaleswaran *et al.* 2016) identified a significant interaction between *FTO* SNP rs8050136 and carbohydrate intake (% energy) ($P_{interaction} = 0.04$), where the 'A' (the high-obesity risk

variant of the *FTO* gene) allele carriers had 2.46 times increased risk of obesity than those with low obesity risk 'CC' genotype ($P = 3.0 \times 10^{-5}$) among individuals in the highest tertile of carbohydrate intake (% energy, mean: 71 %). A significant interaction was also observed between *FTO* SNP rs11076023 and dietary fibre intake ($P_{interaction} = 0.0008$), where individuals with AA genotype in the 3rd tertile of dietary fibre intake had, on average, 1.62 cm lower waist circumference than those with low obesity risk 'T' allele ($P = 0.02$) (**Figure 1a**). A similar but non-significant interaction trend was observed with BMI ($P_{interaction} = 0.03$), where individuals with 'AA' genotype in the 3rd tertile of dietary fibre intake (44 g/day) had, on average, 0.50 kg/m^2 lower BMI scores than those with 'T' allele carriers ($P = 0.07$) (**Figure 1b**). Furthermore, among those who were physically inactive, the 'A' allele carriers of the SNP rs8050136 had 1.89 times increased risk of obesity than those with 'CC' genotype ($P = 4.0 \times 10^{-5}$). In summary, these findings indicate that Asian Indians with at least one copy of the *FTO* obesity-risk allele who consume a high carbohydrate diet or are physically inactive are at particularly high risk of obesity, while high fibre intake may protect against obesity risk in this group.

Given that India leads the world in prevalence of type 2 diabetes and 28–44 % of Asians carry at least one copy of the *FTO* risk allele (Li *et al.* 2012), our study highlights the need to discourage consumption of foods high in sugars and refined carbohydrate and encourage intake of high fibre foods and increased physical activity levels, as following such advice could substantially reduce the genetic risk of obesity and type 2 diabetes among Asian Indians.

Impact of genes and diet on postprandial lipaemia in a British population

Postprandial lipaemia is characterised by a rise in triglyceride-rich lipoproteins after eating. The magnitude and duration of the postprandial lipid response have been shown to be highly variable between individuals due to genetic and dietary factors (Perez-Martinez *et al.* 2010; Ordovas 2001). Given that we spend nearly 75% of the time in a postprandial state, with a continual fluctuation in the degree of lipaemia throughout the day, the normal physiological meal intake pattern is likely to be more evident after a sequential meal challenge which more closely mimics habitual eating patterns. Hence, we designed postprandial studies (Jackson *et al.* 2016; Shatwan *et al.* 2016) to examine the effect of two commonly studied candidate gene variants (Fontaine-Bisson *et al.* 2007; Wybranska *et al.* 2003; Lopez-Miranda *et al.* 2004)[tumor necrosis factor-alpha (*TNFA*) -308G → A and Lipoprotein lipase (*LPL*) (rs328, *S447X*) SNPs] on postprandial lipaemia in 261 participants using a novel sequential meal challenge [standard test breakfast (0 min; 3.9 MJ energy, 111 g carbohydrate, 19 g protein and 49 g fat) and lunch (330 min; 2.3 MJ energy, 63 g carbohydrate, 15 g protein and 29 g fat)]. The type of fat contained within the test meals was predominately saturated, with 29 g of saturated fatty acids (SFA) in the breakfast and 14 g of SFA in the lunch.

In our postprandial analysis, a 30% higher incremental area under the curve (IAUC) was observed for the postprandial triacylglycerol (TAG) response in the GG homozygotes of the *TNFA* -308G → A polymorphism than A-allele carriers ($P = 0.004$) and the genotype explained 19% of the variation in the incremental area under the curve (IAUC) (**Figure 2**). Stratification by sex showed that the genetic associations on TAG IAUC were significant only in men ($P = 0.03$) and not women ($P = 0.39$) (Jackson *et al.* 2016). For the *LPL* polymorphisms, there was an association of the *S447X* polymorphism with postprandial TAG and glucose levels, where *S447* homozygotes had 12% higher TAG area under the curve (AUC) ($p = 0.037$), 8.4% higher glucose-AUC ($p = 0.006$) and 22% higher glucose-

incremental area under the curve (IAUC) (p = 0.042). A significant gene–sex interaction was observed for TAG-AUC ($P_{interaction}$ = 0.004) (**Figure 3a**) and TAG-IAUC ($P_{interaction}$ = 0.016) (**Figure 3b**), where associations were only evident in men (Shatwan *et al.* 2016). In summary, our findings show that postprandial triglyceride levels are influenced by the presence of GG genotype of *TNFA* SNP and *S447* homozygous genotype of *LPL* SNP in men.

These postprandial studies provided novel findings of the effect of diet and genes on postprandial lipaemia in a British population suggesting the role of TNFA and LPL in postprandial lipaemia. Further studies are warranted to investigate the mechanisms underlying the effect of these genes on postprandial lipid metabolism.

Impact of genes and diet on metabolic diseases in other developing countries

Several genes have been identified through candidate gene studies (Vimaleswaran *et al.* 2012b) and genome-wide scans for metabolic diseases (Willer *et al.* 2009; Vimaleswaran & Loos 2010) in Western populations and the interactions of these genes with dietary factors have also been explored (Du *et al.* 2011; Vimaleswaran *et al.* 2012a). However, in several developing countries, nutrigenetics studies have not been carried out because expertise, infrastructure and funds are limited. As part of the *GeNuIne Collaboration*, nutrigenetics studies are being implemented in developing countries including Brazil, Morocco, Thailand, Indonesia, Sri Lanka and Pakistan, for which plans of designing large population-based studies are underway. In addition, workshops on nutrigenetics and nutrigenomics are also being conducted supported by funds from local organisations in developing countries, the British Council and Newton funds to mediate knowledge- and technology- transfer to the developing countries.

From nutrigenetics to personalised nutrition

It is becoming increasingly clear that genes and dietary factors can significantly influence the risk of developing metabolic diseases (Vimaleswaran & Loos 2010; Vimaleswaran *et al.* 2012b). Although many genetic polymorphisms have been identified for metabolic diseases using candidate gene (Vimaleswaran *et al.* 2012b) and genome-wide association studies (Bradfield *et al.* 2012; Willer *et al.* 2009), it has been shown that these variants do not cause these diseases without an obesogenic dietary exposure (Rhee *et al.* 2012). While advances in the field of high throughput genetic analysis have shown the contribution of SNPs to metabolic diseases, the molecular and pathophysiological mechanisms underlying these SNP-diet interactions remain unexplored. Functional studies are required to understand their biological significance and potential clinical application. Besides genes and diet, the gut microbiota and gene-diet-microbe interactions can also modify the risk of developing metabolic diseases (Hu 2011; Qi & Cho 2008). Hence, the combined application of nutrigenetics and nutrigenomics with molecular and metabolite profiling to define an individuals' metabotype will be required to provide the basis for implementing personalised nutrition for metabolic disease prevention.

In summary, the GeNuIne Collaboration supported by the BNF Drummond Pump Priming award is the first to provide an evidence for a gene-diet (nutrigenetics) and gene-physical activity interaction on obesity traits in an Asian Indian population. Further understanding of how *FTO* gene influences obesity related outcomes through dietary and exercise interventions is warranted to advance the development of behavioral intervention and personalised lifestyle strategies, which could reduce the risk of metabolic diseases in this

Asian Indian population. I am also implementing nutrigenetics studies for the first time in other developing countries such as Brazil, Morocco, Turkey, Thailand, Sri Lanka, Indonesia and Pakistan as part of the on-going GeNuIne Collaboration. Nutrigenetics findings from these studies are essential in terms of identifying the genetic heterogeneity in gene-nutrient interactions across various ethnicities and also personalising diet according to each ethnic sub-group.

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GeNuIne Collaborators

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Conflict of interest

The author has no conflicts of interest

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Figure legends

Figure 1a: Interaction of the *FTO* gene polymorphism (rs11076023) with dietary fibre intake on waist circumference in Asian Indians (Vimaleswaran *et al.* 2016). The individuals with AA genotype who are in the 3rd tertile of dietary fibre intake have 1.62 cm smaller waist circumference compared to those with 'T' allele carriers (P = 0.02).

Figure 1b: Interaction of the *FTO* gene polymorphism (rs11076023) with dietary fibre intake on body mass index in Asian Indians (Vimaleswaran *et al.* 2016). The individuals with AA genotype who are in the 3rd tertile of dietary fibre intake have 0.50 kg/m^2 lower body mass index compared to those with 'T' allele carriers (P = 0.07).

Figure 2: Incremental triacylglycerol response (mmol/l) according to the *TNFA* -308 G/A polymorphism (Jackson *et al.* 2016). Mean (SEM) for the incremental triacylglycerol response (mmol/l) according to the *TNFA* -308 G/A polymorphism [GG genotype (N= 162), open squares and GA+AA genotype combination (N= 64) open circles] after consumption of a test breakfast (49 g fat) at 0 min and a test lunch (29 g fat) at 330 min. P value represents the difference in the incremental triglyceride response between the genotypes (GG vs. GA+AA) of the *TNFA* -308 G/A polymorphism.

Figure 3a: Mean (SEM) for the area under the curve (AUC) triacylglycerol response according to *S447X* polymorphism after consumption of a test breakfast (49 g fat) at 0 min and a test lunch (29 g fat) at 330 min. *S447* homozygotes (n=213) had 12% higher triacylglycerol area under the curve (AUC) (P=0.037) compared to *447X* carriers (n=48) for men (Shatwan *et al.* 2016). Carriers of one or two copies of 447X minor allele are combined and presented by white bars. Gene-sex interaction was statistically significant for area under the TAG curve values ($P_{interaction}$=0.004).

Figure 3b: Mean (SEM) for the incremental area under the curve (IAUC) triacylglycerol response according to *S447X* polymorphism after consumption of a test breakfast (49 g fat) at 0 min and a test lunch (29 g fat) at 330 min in men and women (Shatwan *et al.* 2016). Carriers of one or two copies of 447X minor allele are combined and presented by white bars. Gene-sex interaction was statistically significant for IAUC triacylglycerol ($P_{interaction}$=0.016).